Designing of a variable frequency standalone impedance analyzer for *in vitro* biological applications

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

Master of Technology

in

Biomedical Engineering

SAIKAT SAHOO

212BM1355

Under the Supervision of

Dr. Kunal Pal
(Project Supervisor)

Department of Biotechnology and Medical Engineering

National Institute Of Technology Rourkela

Rourkela, Odisha - 769008, India

June 2014

Department of Biotechnology and Medical Engineering

National Institute of Technology Rourkela

Rourkela, Odisha-769008, India.

Certificate

This is to certify that the thesis entitled "**Development of portable standalone impedance measuring device for *in vitro* applications**" by **Saikat Sahoo (212BM1355),** in partial fulfillment of the requirements for the award of the degree of Master of Technology in Biotechnology Engineering during session 2012-2014 in the Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela is an authentic work carried out by him under our supervision and guidance. To the best of our knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Dr. Kunal Pal
(Guide)
Assistant Professor

Acknowledgement

Successful completion of this project is the outcome of consistent guidance and assistance from many people, faculty and friends and I am extremely fortunate to have got this all along the completion of the project.

I owe my profound gratitude and respect to my project guide **Prof. Kunal Pal**, Department of Biotechnology and Medical Engineering, NIT Rourkela for their invaluable academic support and professional guidance, regular encouragement and motivation at various stages of this project. Special thanks to **Prof. Indranil Banerjee** for giving such beautiful ideas and co-operation for the work. I am very much grateful to them for allowing me to follow my own ideas.

It is a privilege to express my profound indebtedness deep sense of gratitude and sincere thanks to Head of Department **Prof. K. Pramanik** for her constant encouragement.

I would like to extend my heartfelt gratitude to research scholars Mr. Biswajeet Champaty, Mr. Sai Satish Saigiri, Mr.Vikay Kumar Singh, Mr. Prashant Dubey Ms Sitiprgyan Satpathi and Ms. Beauty Behera whose ever helping nature and suggestions have made my work easier by many folds.

I would like to thank all my friends and classmates for their constant moral support, suggestions, advices and ideas. I have enjoyed their presence so much during my stay at NIT, Rourkela.

I am also grateful to NIT Rourkela for providing me adequate infrastructure, experimental facilities to carry out the present investigations. I acknowledge all staffs, research scholars,

friends and juniors of Department of Biotechnology And Medical Engineering, NIT, Rourkela for helping me during my research work.

Last but not least I would like to thank my mother, who were always caring for me throughout my journey till date. They rendered me enormous support during the whole tenure of my stay at NIT Rourkela.

SAIKAT SAHOO
212BM1355
Biotechnology and Medical Engineering
National Institute of Technology Rourkela,
Odisha-769 008 (India)

Contents

List of figures

List of tables

Abstract

Maximum biological samples have some electrical property, which gave us a new dimension in the field of biomedical engineering. Now-a-days measurement of impedance by applying an electrical voltage/current, has a broader application for analyzing different biological samples. Most of the devices used for the measurement of bio-impedance are bulky and much costlier. This approach will help us to design a portable, standalone, multi frequency (10Hz – 10kHz) bio-impedance monitoring device with acceptable accuracy and resolution for in-vitro studies of biological cells and tissue.

Keywords: Bioimpedance, constant current source, polarization, reactance

Chapter I

Introduction and Objectives

1.1 Introduction to bio-impedance

Impedance is an electrical property of a material, for biological samples the same property is called bioimpedance. Resistance, capacitance and inductance are the three components of impedance. Impedance can be defined as the opposing force to the flow of electron for a material or the opposing force against the flow of ions in a solution. [1] For a biological sample there is a resistive part and a capacitive part, The resistive part responds for both in ac and dc, but the capacitive part responds only in ac supply. For ac characteristic the impedance is expressed as a combination of two parts resistance (R) and reactance (X) (capacitive resistance), where resistance is the real part and capacitance is the imaginary part. Thus impedance can be expressed a complex quantity, Z. In polar form, Z is expressed as $Z = |Z|e^{j\theta}$, where magnitude |Z| represents the ratio of the voltage amplitude and current amplitude and θ represents the phase difference between voltage and current. [2] It Cartesian form it can be also represents as Z = R + jX.

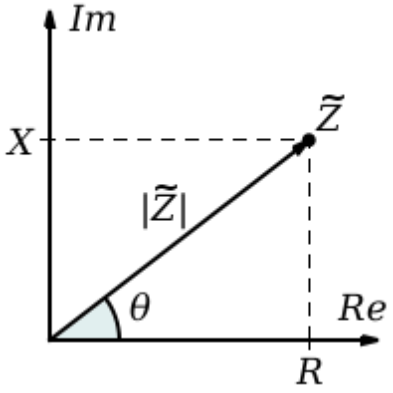

Figure 1.1 Graphical representation of complex impedance [2]

Cells can be modeled as a group of electrical components. The extracellular space is represented as a resistor (Re), and the intracellular space and the membrane is modeled as a resistor (Ri) and

a capacitor (Cm) Figure 2. Both the extracellular space and intracellular space are highly conductive, because they contain salt ions. The lipid membrane of cells is an insulator, which prevents current at low frequencies from entering the cells. At lower frequencies, almost all the current flows through the extracellular space only, so the total impedance is largely resistive and is equivalent to that of the extracellular space. As this is usually about 20% or less of the total tissue, the resulting impedance is relatively high. [3] At higher frequencies, the current can cross the capacitance of the cell membrane and so enter the intracellular space as well. It then has access to the conductive ions in both the extra and intra-cellular spaces, so the overall impedance is lower Figure 3(b).

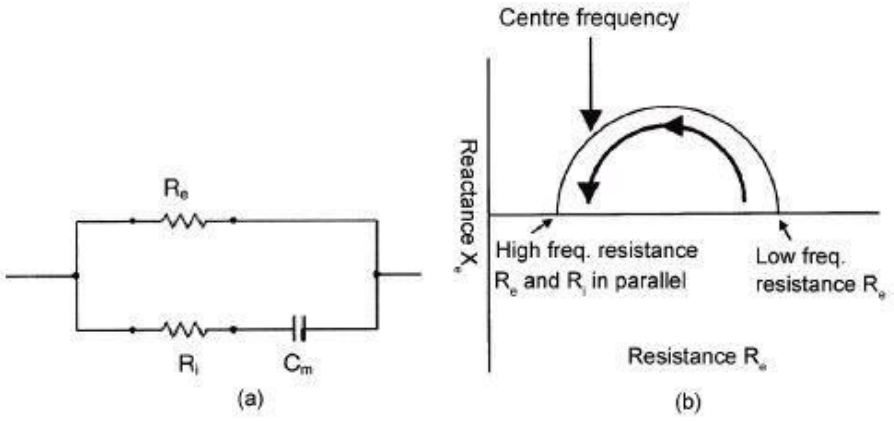

Figure 1.2 (a) The cell modelled as basic electronic circuit. Ri and Re are the resistances of the intracellular- and extracellular-space, and Cm is the membrane capacitance. (b) Cole–Cole plot of this circuit. [4]

The movement of the current in the different compartments of the cellular spaces at different frequencies, and the related resistance and reactance values measured, is usefully displayed as a Cole–Cole plot. [4] This is an extension of the resistance/reactance plot in the complex plane. Instead of the single point for a measurement at one frequency, the values for a range of frequencies are all superimposed. For simple electronic components, the arc will be a semicircle

(Figure 2(b)). At low frequencies, the measurement is only resistive, and corresponds to the extracellular resistance, no current passes through the intracellular path because it cannot cross the cell membrane capacitance. As the applied frequency increases, the phase angle gradually increases as more current is diverted away from the extracellular resistance, and passes through the capacitance of the intracellular route. At high frequencies, the intracellular capacitance becomes negligible, so current enters the parallel resistances of the intracellular and extracellular compartments. The cell membrane reactance is now nil, so the entire impedance again is just resistive and so returns to the X axis. Between these, the current passing through the capacitive path reaches a peak. The frequency at which this occurs is known as center frequency (Fc) and it

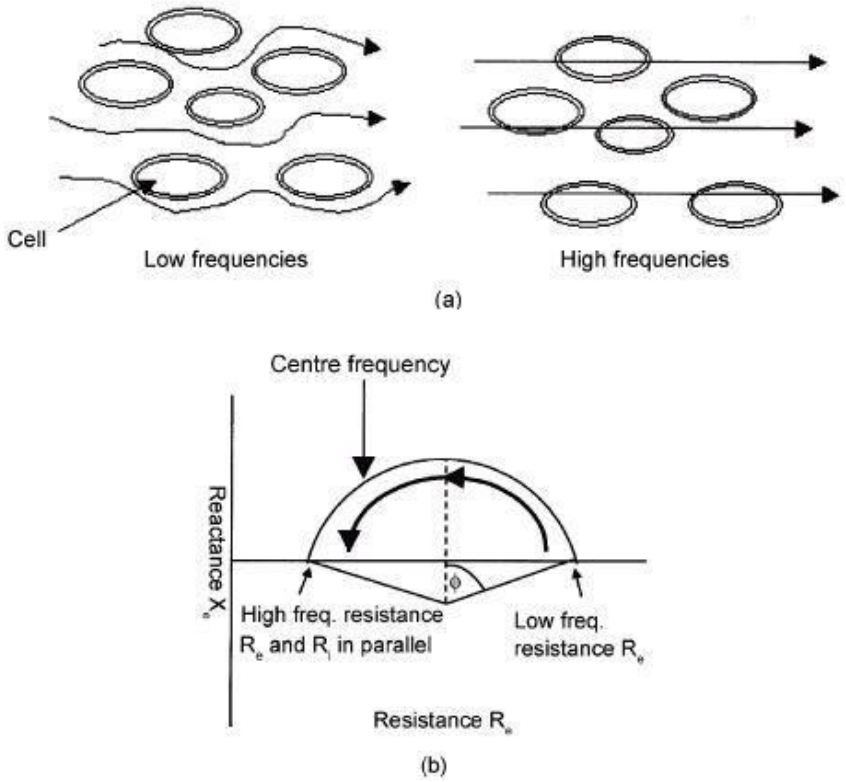

Fig. 1.3 (a) The movement of current through cells at both low and high frequencies. (b) Idealized Cole–Cole plot for tissue [4]

is a useful measure of the properties of an impedance. In real tissue, the Cole–Cole plot is not exactly semicircular, because the detailed situation is clearly much more complex; the plot is usually approximately semicircular, but the centre of the circle lies below the x-axis. [5] Inspection of the Cole–Cole plot yields the high and low frequency resistances, as the intercept with the x-axis, and the centre frequency is the point at which the phase angle is greatest. The angle of depression of the centre of the semicircle is another means of characterizing the tissue. [4]

1.2. Application of bioimpedance analysis

From the above discussion it is clear that electrical bio-impedance carries information about the physiological performance of living tissue. Bio-impedance based methods have obtained a recognized position among the clinical methods of non-invasive diagnosis of cardiovascular system. Now-a-days bio impedance methods have already replaced the EMG based diagnosis systems because of its accuracy and good performance. [6] Bio-impedance is also an important measurement for iontophoretic drug delivery, non-invasive method for penetration of ionized drug through skin. [7] The above all are in-vivo applications of bio-impedance. There are also lots of applications of bio-impedance method for in-vitro analysis.

1.3 Objectives

The prime aim of our work is to design a portable, stand alone bioimpedance monitoring device for in-vitro applications. This work includes:

1. Development of a portable impedance analyzer using microcontroller and suitable electronic circuit.

2. To measure the accuracy and resolution of the developed bio-impedance analyzer.
3. Study the impedance profile of some biological samples like goat blood, goat fat, *E. coli* bacteria etc.

Chapter II

Literature Review

2.1. Measurement of bio-impedance

In recent years, there has been an increased attention towards analysis of the physiology of the cells (mammalian or microbial) and tissues by measuring the electrical properties. [8] The cells and the tissues show higher impedances at lower frequencies and corresponding lower impedances at higher frequencies suggesting capacitor like behavior. This may be attributed to the dielectric material like behavior of the cell membranes. Due to this kind of behavior, most of the modeling of the cells and the tissues has been reported having the same characteristic as R-C combinations. [3] Many research groups are working towards measuring the growth and physiology of the cells by measuring impedance magnitude or phase. [9]. Unfortunately, the devices available for the study are quite costly.

Bio-impedance can be measured with a constant current source mechanism. The current is kept constant for any load variation and the output voltage is accrued across the load. We can calculate the modulus of impedance (|Z|) by simply divide the measured output voltage by the constant current.

$$|Z| = V_{rms} / I_{rms} ;$$

Whwer, V_{rms} is the root mean square (rms) value of output voltage

I_{rms} is the rms value of constant current

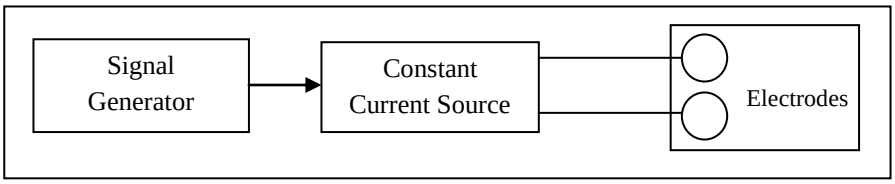

Figure 2.1 General block diagram of constant current source based impedance analyzer

2.2. Constant current source

Bio-impedance can be measured using constant current source. There are many constant current sources like howland current source, current mirror based constant current source, but application of these current sources are limited to lower value of frequency and impedance. The impedance of biological samples is basically high in the range of 10 kΩ to 1MΩ. So the mentioned currents sources do not provide a constant current in the specified range. Voltage controlled constant current source (VCCS) is a simple method for maintaining a constant current up to a range of 3MΩ. Due to its simplicity, stability and other advantages it has been used in bioimpedance analysis (BIA) for tissue characterization.

2.2.1. Howland constant current source

Howland constant current source is widely used in electronic circuits to maintain a constant flow of current. It is configured by simple operational amplifier as shown in Figure 2.2. There are different arrangements of howland current source but the concept for generating constant current is same for all the cases. Its working frequency range is around 10 Hz to 100 kHz. But in case very high load >10 kΩ the current value starts decreasing. It is also a VCCS type constant current source. For maintaining constant current there should be a match between resistors R1 = R3 and R2 = R4. Practically, it is not possible to have two resistors of exactly equal value, this limitation debarred the use of Howland constant current source.

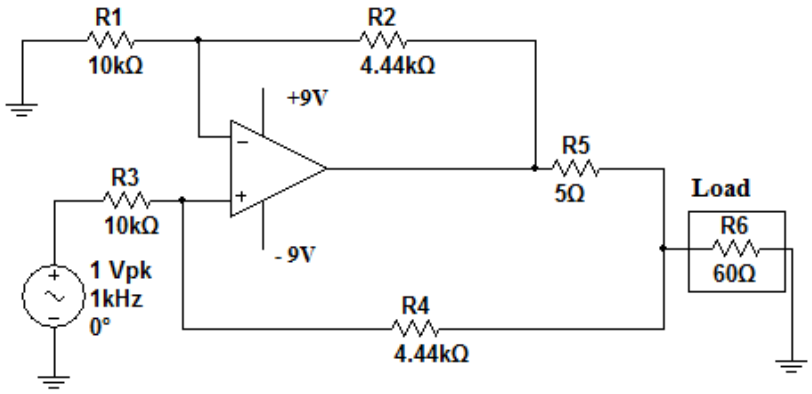

Figure 2.2 Howland constant current source configuration [10]

2.2.2 Current mirror based constant current source

Current mirror is used to maintain equal current flow in two different branches regardless of loading. This type of current source is more advantageous because of its high input impedance. For generation of constant current we need a current generator, current mirror and a current regulator. This types of current sources are also called current control current source (CCCS).The accuracy and precision is better compared to VCCS.

Current mirror type constant current sources also have limitation on high impedance, that's why it is not used for bio-impedance analysis (BIA). [11]

2.2.3 VCCS with inverting amplifier mode

Among the VCCSs, non-inverting amplifier type constant current source is the simplest, accurate and stable design for maintaining a constant current up to a range of 2MΩ load resistance. For these features this type of current sources are widely used for medical applications like bioimpedance analysis. [12]

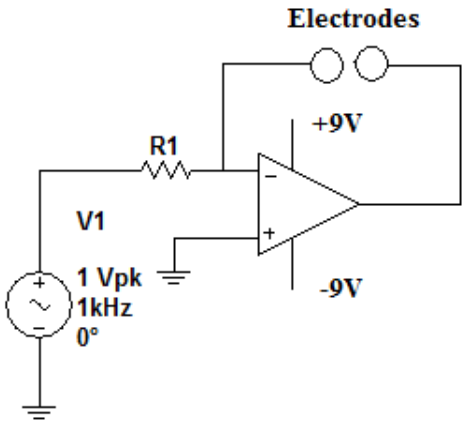

Figure 2.3 Voltage controlled constant current source using inverting amplifier

2.3 Arduino microcontroller

Arduino is an complete package of modern microcontroller board interfaced with required electronic devices (ADC, DAC, LEDs), with better flexibility and user friendliness. These types of microcontrollers are very user friendly and robust to interface with different electronics circuitry, capable of taking direct analog output, easy to upload programs by USB port. Arduino boards are USB powered or it can be driven by dc battery supply or ac to dc adaptor of 5V, 1A voltage rating. All the arduino based microcontrollers are flexible, easy to use, user friendly and some of them have the advantage of interfacing with GSM network, TCP-IP network, android phones also with Bluetooth, Wi-Fi facility.

2.3.1 Arduino DUE / Duemolive

Arduino DUE is a special version of arduino group with an inbuilt digital to analog converter (DAC) for converting the digital data into analog form. It is configured with 32 bit ARM microcontroller with a clock frequency of 84 MHz. Arduino DUE has 54 digital input/output

pins of which 12 can be used as PWM (pulse width modulation) output, 12 analog input pins, 2 DAC output pins (DAC0 & DAC1) etc. The resolution of the inbuilt DAC is maximum 12 bit, we can also use it in 8 bit or 10 bit resolution by simply changing the program.

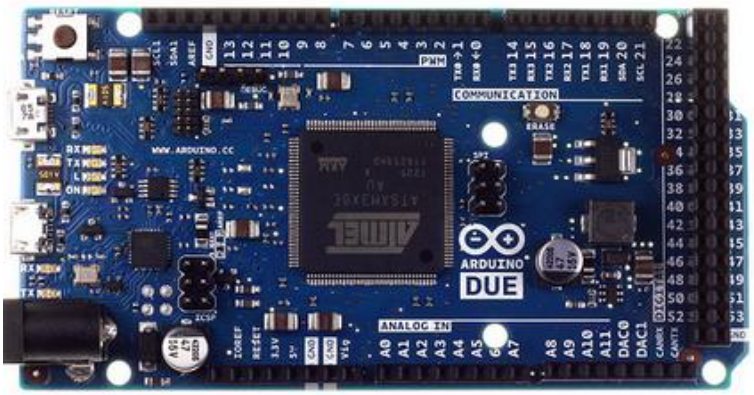

Figure 2.4 Top view of Arduino DUE microcontroller board

2.3.2. Arduino MEGA ADK

Arduino MEGA ADK is another special version of arduino based on ATmega2560 microprocessor chip. Special feature of microcontroller board is USB interface to connect with android based phones. Further it has 54 input/output pins, 16 analog input pin same like arduino DUE with a crystal oscillator of 16MHz.

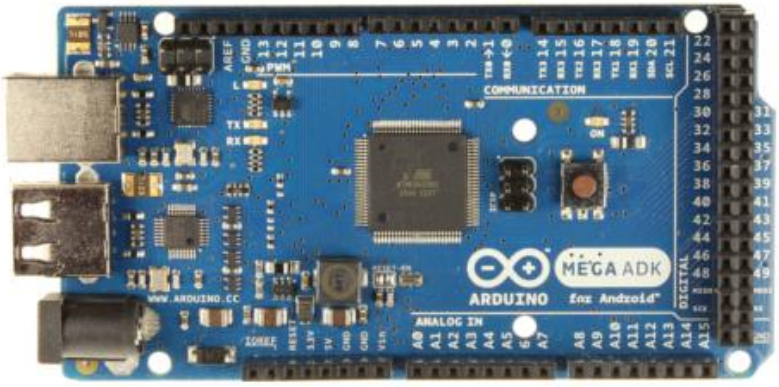

Figure 2.5 Arduino MEGA ADK hardware kit

2.4. Operational amplifier (OP07)

OP07 is an operational amplifier IC with 8 pins for input and output, where pin 2 and 3 are for inverting and non-inverting input, pin 4 and 7 are for –ve and +ve power supply, pin number 6 is used for output (Figure 2.6). Operational amplifier can be used as a voltage amplifier, comparator or switching purpose. It has high input impedance and low output impedance. It works on two amplifying mode, inverting and non-inverting with a high gain factor (10^5). High CMRR of OP-07 is about 126 dB. [13]

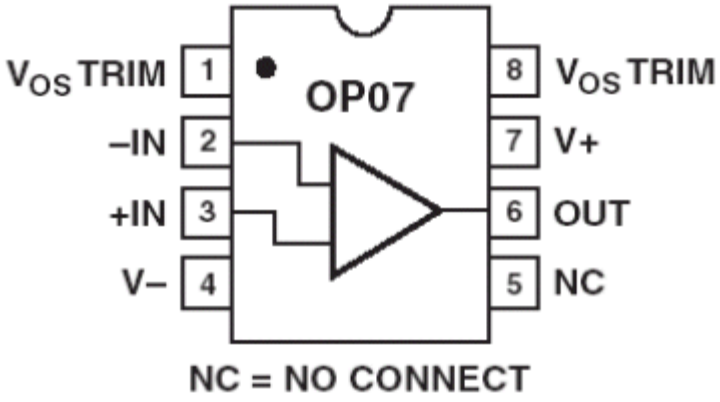

Figure 2.6 Pin diagram of IC OP07

2.5. Passive High pass and low pass filters

Filters designed by resistor, capacitor or inductor are called passive filter because this type of filters does not depend upon external power source. A simple first order low pass filter can be designed by RC circuit as shown in Figure 2.7, in this figure if we interchange the position of resistor (R) and capacitor (C) then it performs as a high pass filter with same cutoff frequency. High pass filter is used to block low frequency, also helps to remove the DC component from the signal. Low pass filter rejects high frequency component, it also used as integrator for specific

condition. As we increase the order of the filter the cut-off will be sharper but the complexity of the circuitry will also increase. We have to design a filter as per the requirement of the circuit.

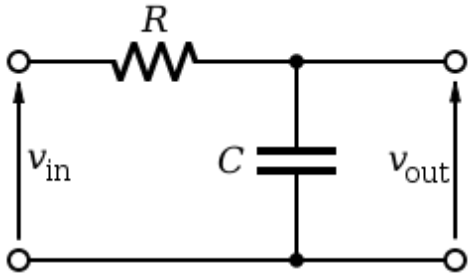

Figure 2.7 Passive RC 1st order low pass filter

The time constant of the filter, T = RC. So, the cutoff frequency of the filter is, $f_c(Hz) = 1/(2\prod RC)$.

2.6. Non-inverting amplifier

Non-inverting amplifier is used to amplify the gain of input signal without changing the polarity of the signal rather we can say without any phase shifting. We can adjust the gain of the amplifier by changing the feedback resistor. Non-inverting amplifier with specific configuration also provides high input impedance, which is advantageous for any biomedical application. [14]

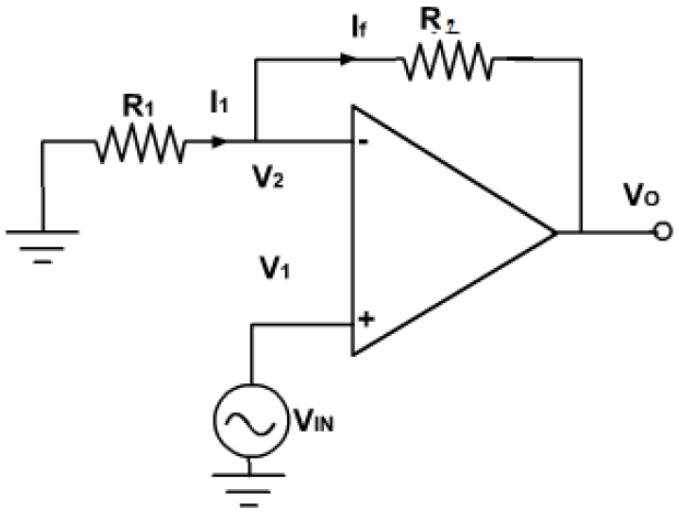

Figure 2.8 Gain of the amplifier is calculated as (V_0 / V_{IN}) = (1+R_2/R_1).

2.7. 16x2 LCD display (JHD 162A)

Liquid Crystal display (LCD) is very commonly used for displaying device with electronic circuit. 16 x 2 LCD display means it can display 16 characters in a row and it contains two such rows. LCDs are the best option compared to seven segment display because LCDs are commercial, easy to program and able to display special characters, custom characters.

LCDs contain two characters for programming, command register and data register. Command register stores the command from master/microcontroller and data register stores the value (ASCII) which is displayed over the LCD.

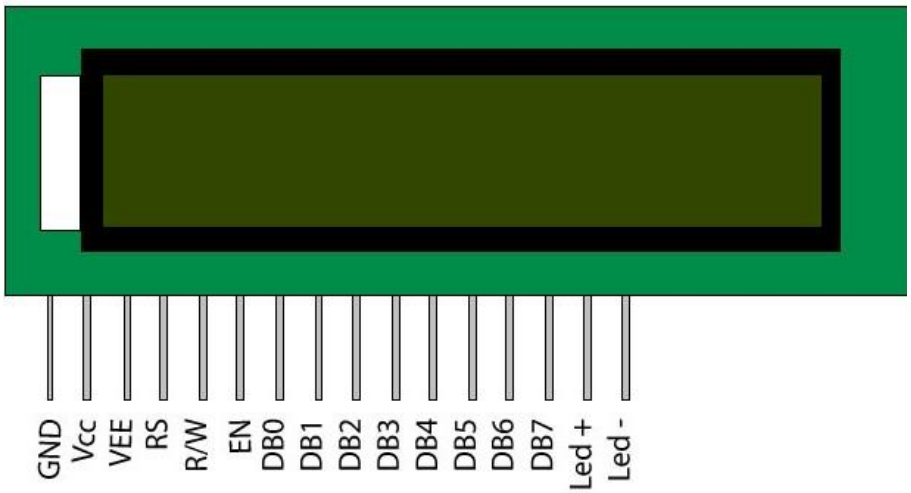

Figure 2.9 16 x 2 LCD display with pin diagram

2.8. 4x4 Matrix keypad

Matrix keypads are also commonly used tool used with the electronic circuit. 4 x 4 matrix keypad means it contains 4 rows and 4 columns which has total 16 switches to input 16 different data.

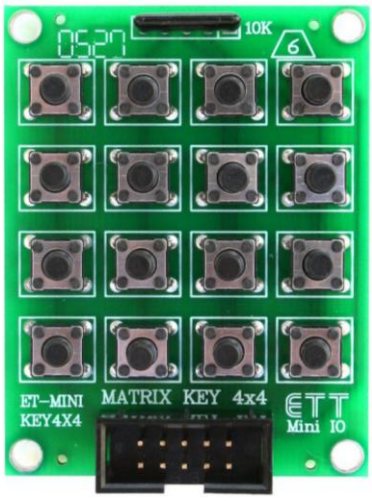

Figure 2.10. 4 x 4 matrix keypad

Chapter III

Materials and methods

3.1. Materials

Arduino microcontrollers (DUE, MEGA ADK) from Arduino, was used for variable frequency signal generator and for signal processing and calculation. Basic electronic components like potentiometer (50 kΩ, 20 kΩ), capacitor (0.01 μF, 10 μF), resistance (1 kΩ, 2.7 kΩ) was collected from local market. Operational amplifier IC (UA741CN) from Texas Instruments, 4x4 matrix keyboard from Robokits and 16x2 LCD display (JHD 162A) were used in the study. Two +9V batteries (Eveready) were used as ±9V power supply of the operational amplifier and a USB adaptor of voltage rating 5V, 1A was used to supply the Arduino microcontrollers. For in-vitro analysis we have cultured *E. coli* bacteria in laboratory and goat blood, goat eye, goat fat was collected from local butcher shop. The number of blood and *E-coli* cells present in the solution under observation was counted in an automatic cell counter (Countess automated cell counter, USA).

3.2. Methodology

The whole set-up consists of signal generator, constant current source and display unit. Signal generator is based on a microcontroller program. To change the frequency of the generated sine wave, matrix keyboard is used. There is a display at the input side which shows the frequency selection menu and another LCD to show the measured impedance and the peak value of the output voltage.

3.2.1. Signal generator

To generate sinusoidal signal by microcontroller, first we have to prepare a look up table in which the digital value of sinusoidal wave in different angle interval was stored. Higher the angel

interval, lower the sampling rate. For a good quality sine wave generation, the number of samples/sampling rate should be higher. The table is designed for only single period of sine wave and then it is repeated in a loop to generate continuous sine wave. By incorporating time delay in the above mentioned loop, we can change the frequency of the sine wave. An analog to digital converter (ADC) is needed to convert the digital bits into a sinusoidal signal. An ADC with 12 bit resolution is incorporated with Arduino DUE, microcontroller we have used. The generated sine wave is a digital or staircase version of original sine wave. An integrator is used to smooth the digital sine wave. The output of integrator is a sine wave of analog nature but it contains some dc offset. To eliminate the dc offset a high pass filter is used with a cut-off frequency of 16 Hz. Different frequency sinusoidal wave was generated by changing the loop delay in the program.

3.2.2. Constant current source

Impedance is measured with constant current source method. [15] The circuit current is always constant for any value of load. This can be done by simple buffer and an inverting amplifier. A variable potentiometer of 20kΩ was used in the feedback path of non-inverting amplifier to adjust the amplitude of generated sine wave. R_s is the fixed series resistance or current controlling resistance. The series resistance R_s is changed only if we want to change the constant current value or in case of the saturation of the output voltage. V_f is the output voltage across the sample. Non-inverting amplifier in the first stage used to provide high input impedance and to adjust the amplitude of the generated sine wave. The high input impedance helps to protect any unwanted current to the signal generator and also protects any noisy signal to disturb the flow of constant current. To supply to the op-amps a ±9V battery combination was used.

3.2.3. Display unit

This unit contains a microcontroller (Arduino) and a LCD display. The absolute value of impedance is calculated by simple ohms law. V_f is the output signal across the sample. I_f is the rms value of constant current, now the impedance can be calculated by ohms law ($|Z| = V_f / I_f$). We have to keep in mind that the output of the constant current source should not saturate. We can control the saturation of the output voltage by controlling the amplification factor of the inverting amplifier. But the microcontroller can read an analog voltage maximum up to +5V, we have to keep in mind that the peak value of the output signal (V_f) should not reach the maximum value (+5V). For that the peak value of output voltage (V_f) is displayed over the LCD. To maintain the output voltage below +5V we have to change the series resistance R_S.

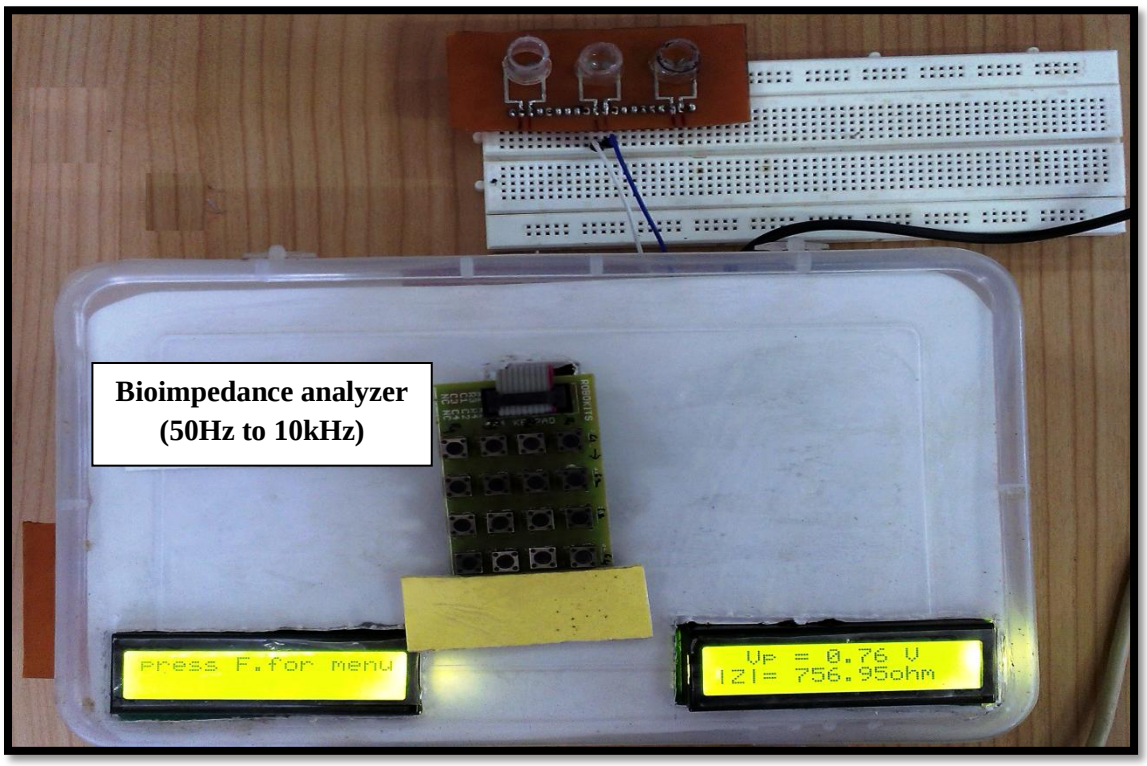

Figure 3.1 Complete setup of developed bioimpedance analyzer

Chapter IV

Results and Discussion

4.1. Stability of constant current source

The stability of constant current source was verified using some standard resistors available in the market. Stability of two constant current sources were checked (Howland constant current source and VCCS with inverting amplifier mode) was measured by the same way. The result was compared to evaluate the best performance at higher frequencies and higher resistance / impedance. For the Howland current source input supply was a sinusoidal wave of $1V_P$, 100 Hz and for VCCS supply was a 1 V_P , 1 kHz sinusoid.

Table 1. Output voltage and current values for Howland current source

Load resistance (Ω)	Output Voltage / V_{rms} (V)	Output Current / I_{rms} (mA)
20	1.41	70.5
50	3.53	70.6
100	6.91	69.1
200	8.68	43.4
500	9.61	19.22

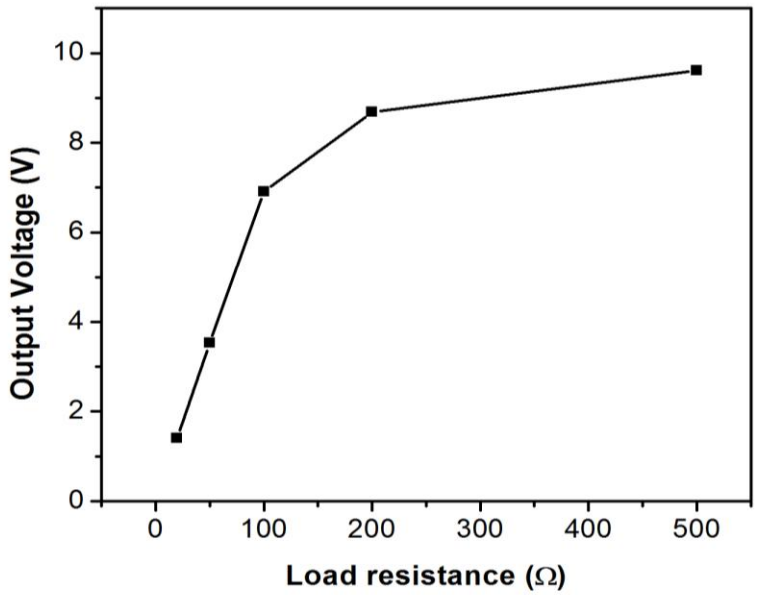

Figure 4.1 Load resistance vs output voltage curve for Howland constant current source

Table 2. Output voltage and current values for VCCS

Load resistance (kΩ)	Output Voltage / V_{rms} (V)	Output Current / I_{rms} (μA)
100	0.707	7.07
200	1.41	7.05
300	2.12	7.06
400	2.83	7.075
500	3.54	7.68
600	4.24	7.06
800	5.66	7.075
1000	6.98	6.89
2000	8.5	4.25

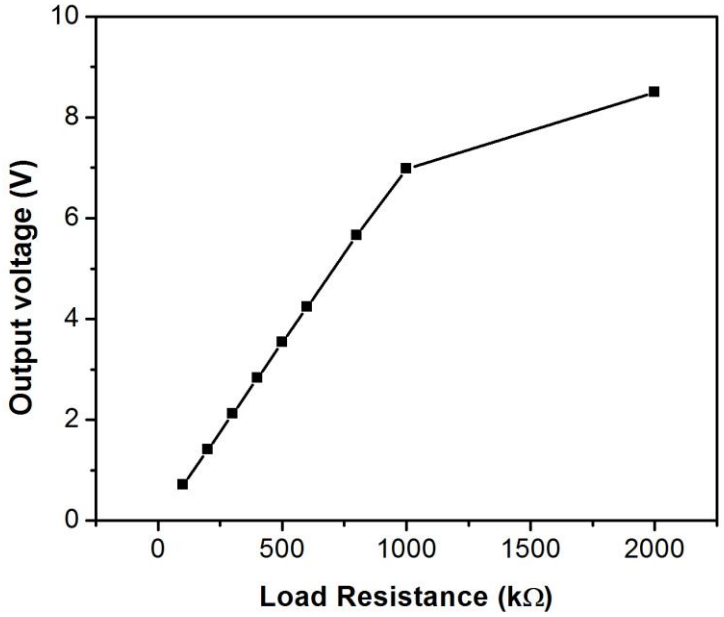

Figure 4.2 Load resistance vs output voltage curve for VCCS based current source.

For the first case the curve shows non-linear behavior after 200 kΩ resistance and for the second case the linear behavior of the circuit deteriorates beyond 1MΩ resistance. The results concluded that that stability of VCCS based constant current source is much better than other constant sources at high load resistance and high frequency. As biological studies deals with high

frequency and high impedance, we preferred VCCS based constant current source for the development of bio-impedance monitoring device.

4.2. Accuracy and Resolution

The accuracy and resolution of the developed circuit is measured using standard resistors. Accuracy is measured for different series resistances (R_S) like 1kΩ, 10kΩ, 56kΩ and 100kΩ. Some standard resistances are connected in place of samples and the corresponding values of resistances are measured by developed impedance analyzer configuration. The true value of the same resistance is measure by a standard multimeter. Accuracy curve is plotted between the true value and measured value for each series resistance. Resolution of the system was measured using standard resistors, adding one by one in series. From the experiment it is observed that the developed system will respond to a minimum 2Ω change in resistance. Which means it can detect any certain change in impedance greater or equal to 2Ω.

4.3. Application of the device

4.3.1. Analyzing hydrogels

Hydrogels are used as the matrix of drugs for ionotophoretic drug delivery purpose. [16] Iontophoretic drug delivery is a non-invasive method to deliver ionized drugs through skin with the application of electrical voltage or current. Skin-electrode interface impedance is very high so application of high voltage is required to force drugs to penetrate skin. Application of high voltage may burn the skin, for this reason generally we use hydrogels as the matrix of drug.[17] Hydrogels are used to reduce the impedance of skin-electrode interface. Higher the impedance of hydrogels lesser the drug will penetrate through skin, so it's important to know the impedance

profile of hydrogels before using it as drug delivery matrix. Four types of hydrogels were prepared and impedance profiles were studied using developed impedance analyzer. We have prepared 10 g of hydrogels with different gum solutions (Guar gum, Acacia gum, Gum ghatti, Xanthan gum). 1% gum solution of above mentioned hydro-gels were prepared. 4g of surfactant mixture (Span 80 and Tween 80 in 1:2 proportions) was mixed properly with 3.5g of sunflower oil and 2.5g of gum solution to prepare final hydrogel. [18]

The impedance profile of four hydrogels was studied by the developed impedance analyzer and plotted (Figure 4.3). After observing Figure 4.3 it is clear that the impedance of the gels are in following order Guar > Acacia > Ghatti > Xanthan. The results were confirmed by microscopic study (Figure 4.4).

Table 3. Impedance chart for 4 different gels

Frequency (Hz)	**Impedance Guar (kΩ)**	**Impedance Acacia (kΩ)**	**Impedance Ghatti (kΩ)**	**Impedance Ghatti (kΩ)**
50	72.45	54.02	39.36	39.24
100	66.58	50.25	38.31	38.73
205	59.88	46.90	37.35	36.39
500	54.86	46.06	36.85	35.46
750	52.76	45.64	36.85	35.00
1000	51.09	44.38	36.51	34.79
2000	48.58	43.96	36.26	34.46
3000	47.74	43.96	36.09	34.21
4000	46.90	43.96	35.92	34.12
5000	46.48	43.96	35.88	34.04
7500	45.64	42.71	35.88	34.00
10000	45.23	40.61	35.67	33.87

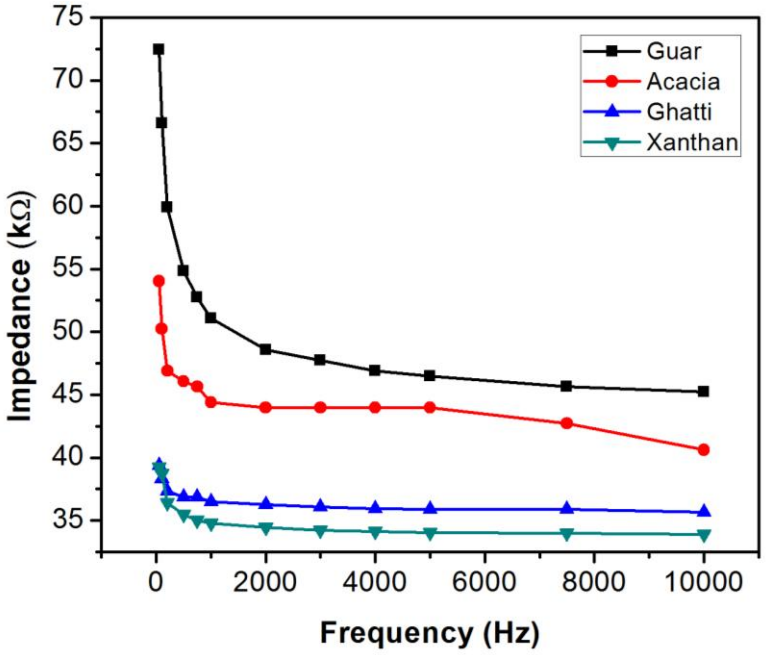

Figure 4.3 Frequency vs. Impedance plot for hydrogels of different composition.

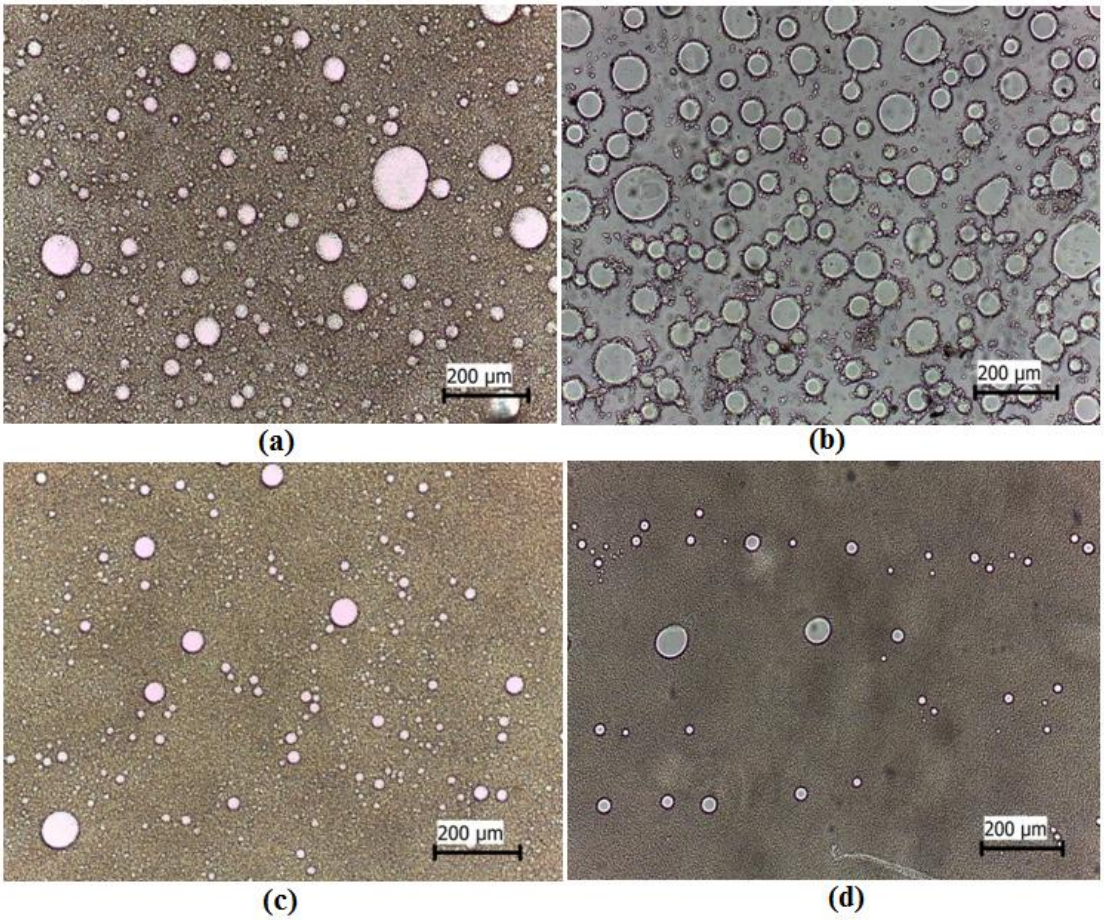

Figure 4.4 Microscopic image for (a) Guar (b) Acacia (c) Ghatti (d) Xanthan based gels

4.3.2 Analysis of blood sample

The impedance profile of goat blood was measured by the developed impedance analyzer in the previously mentioned frequency range. Three types of blood samples are prepared in the proportion mentioned in Table. 4 and the impedance profile was measured for each case. By observing the Figure 4.5 we can clearly see that the impedance is decreasing as we are approaching to the higher frequency. This kind of nature of goat blood confirms the capacitive behavior. Among the three cases the impedance is higher for the pure blood. Blood contains different cells and tissues, more number of cells oppose the flow of current. It means that higher the number of cells higher is the impedance. From Figure 4.5 we can clearly say that |Z1| > |Z2| > |Z3|, where |Z1| is the impedance of pure blood and |Z2| & |Z3| are the diluted version of the blood. As per the theory pure blood contains more number of cells than the other two samples as a result it shows higher impedance than the others.

Table 4. Sample preparation details of three blood samples

Sample	Ratio of blood and saline water
Sample 1 (Z1)	5 : 0
Sample 2 (Z2)	4 : 1
Sample 3 (Z3)	1 : 4

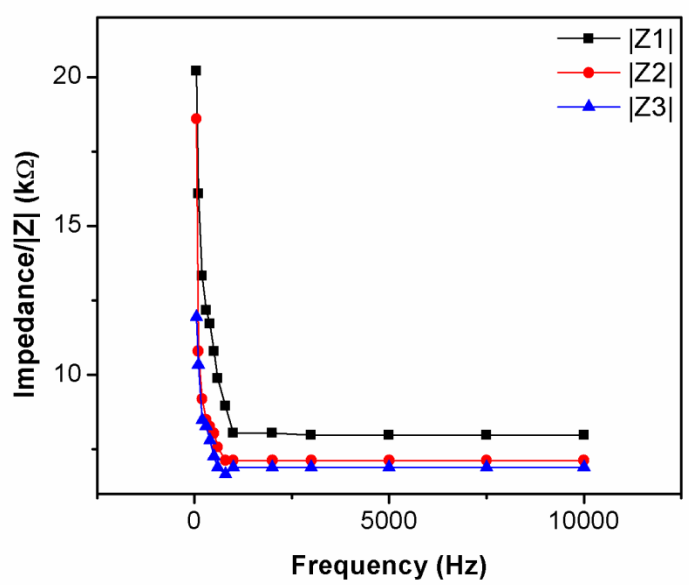

Figure 4.5 Impedance vs. frequency plot for different blood sample

4.3.3. Analysis of *E. coli* suspensions

Cultured *E.coli* was prepared in five different concentrations in PBS (Phosphate Buffer Solution). Sample 1 (Z1) has lowest concentration and sample 5 (Z5) has highest concentration of *E. coli.* To prepare the sample first the cultured *E. coli* solution was centrifuged for 8 min in 5000 rpm. [19] The suspended bacteria for each case was collected after centrifugation and mixed with 5 ml of PBS to prepare the final sample. Impedance profile of five different solutions was measured using the developed impedance analyzer in the frequency range of 50Hz to 10kHz. The graph is plotted to study the change in impedance with respect to change in frequency, shown in Figure 4.6. According to theory, impedance should increase if the number of biological cells increases in the solution. [15] UV spectroscopy was done in 545 nm wavelength to confirm the presence of *E.coli* in different samples. [20] Figure 4.7 shows the plot between the bacterial count and the absorbance value from UV spectroscopy. The linear nature of the curve acknowledges that the concentration of bacteria is increasing linearly in this manner,

Z1 < Z2 < Z3 < Z4 < Z5. Comparing the frequency vs. impedance plot in Figure 4.6 and absorbance curve in Figure 4.7 we can conclude that more the number of bacterial cells present in the solution, the impedance of the solution increases to oppose the flow of current.

Table 5. Sample details of *E. coli* suspended samples

Sample	**Nutrient broth (ml)**	**Cultured *E. coli* solution (ml)**	**Bacterial count (CFU/ml)**
Sample1(Z1)	5	1	675000
Sample2(Z2)	5	2	1.65E6
Sample3(Z3)	5	3	3.3E6
Sample4(Z4)	5	4	4.67E6
Sample5(Z5)	5	5	5.2E6

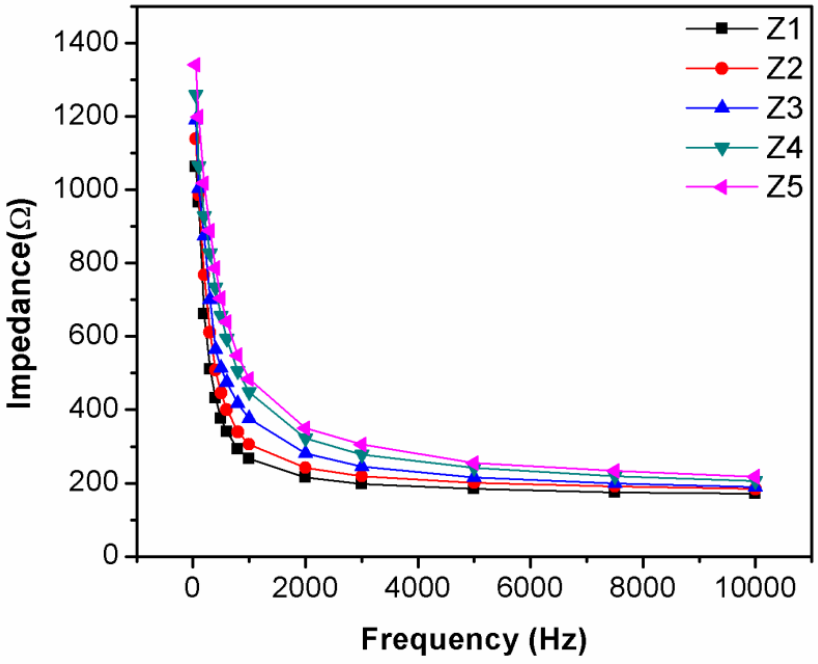

Figure 4.6 Frequency vs. impedance plot for *E. coli* samples in different concentration.

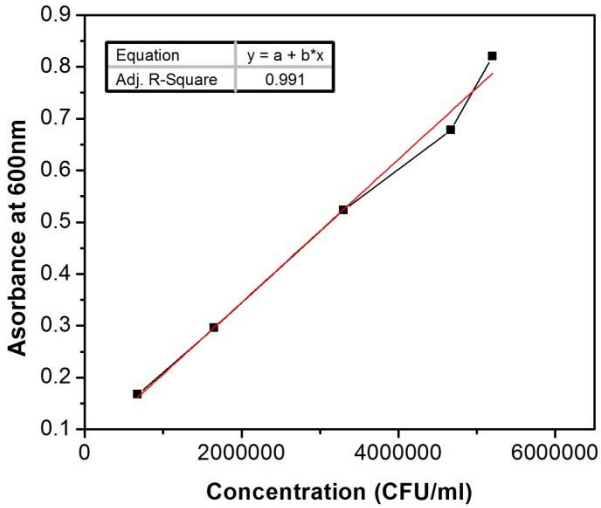

Figure 4.7 Absorbance curve obtained by UV spectroscopy for five samples listed in Table. 5

4.3.4. Analysis of goat fat

Now-a-days many studies are going on the measurement of body fat by measuring bioimpedance. These studies tried to correlate the bioimpedance property with the amount of body fat present in our body. [21] Inspired by those studies we have measure the impedance profile of goat fat using the developed impedance analyzer. The experimental results (Figure.4.8) confirm the capacitive behavior of goat fat.

Table 6. Change in impedance with frequency of goat fat

Frequency (Hz)	Impedance (kΩ)
50	38.15
100	35.74
200	32.8
300	31.82
400	30.35
500	29.37
600	28.88
800	27.9
1000	27.41
2000	27.41
3000	26.92
5000	26.43
7500	26.43
10000	26.43

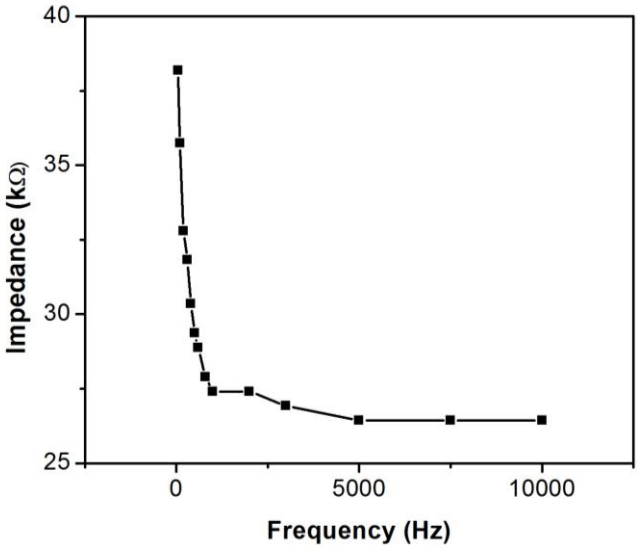

Figure 4.8 Impedance profile of goat fat.

4.3.5. Analysis of vitreous humour of goat eye

Vitreous is a gel type substance that fills the gap between lens and retina. Goat eye was dissected to extract the vitreous humour for impedance analysis. It was put inside the electrode configuration to measure the impedance (Figure 4.9). Significance of this study is the difference between impedance profile of vitreous humour of normal eye and diseased eye. Thus impedance can be a major parameter for detecting eye diseases.

Table 7. Impedance values of vitreous humour measured in variable frequency

Frequency (Hz)	Impedance (Ω)
50	761.07
100	722.7
200	634.14
300	576.26
400	542.07
500	507.89
600	478.59
800	439.52
1000	419.99
2000	380.92
3000	366.27
5000	332.31
7500	327.08
10000	307.66

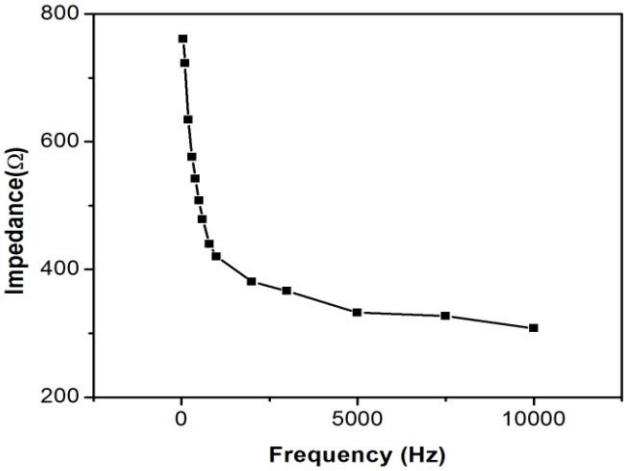

Figure 4.9 Impedance characteristic of vitreous humour of goat eye

4.4. Continuous monitoring

Continuous monitoring was done by interfacing the impedance analyzer unit (Arduino MEGA ADK) with Matlab. A program was designed in Matlab to plot graph between time and impedance value. The fluctuation of the impedance value with respect to time can be tracked by continuously monitoring the impedance of the sample. Figure 4.10 shows the graph for

continuous monitoring of pure goat blood and its two diluted versions (Figure 4.11 & 4.12), taken for each 100 seconds. Pure blood was diluted by normal saline in a proportion of 4:1 (pure blood : saline) and 1:4 ((pure blood : saline). If we clearly observe the three graphs, we can see that there is a decrease in the impedance value for the diluted samples with respect to the pure blood.

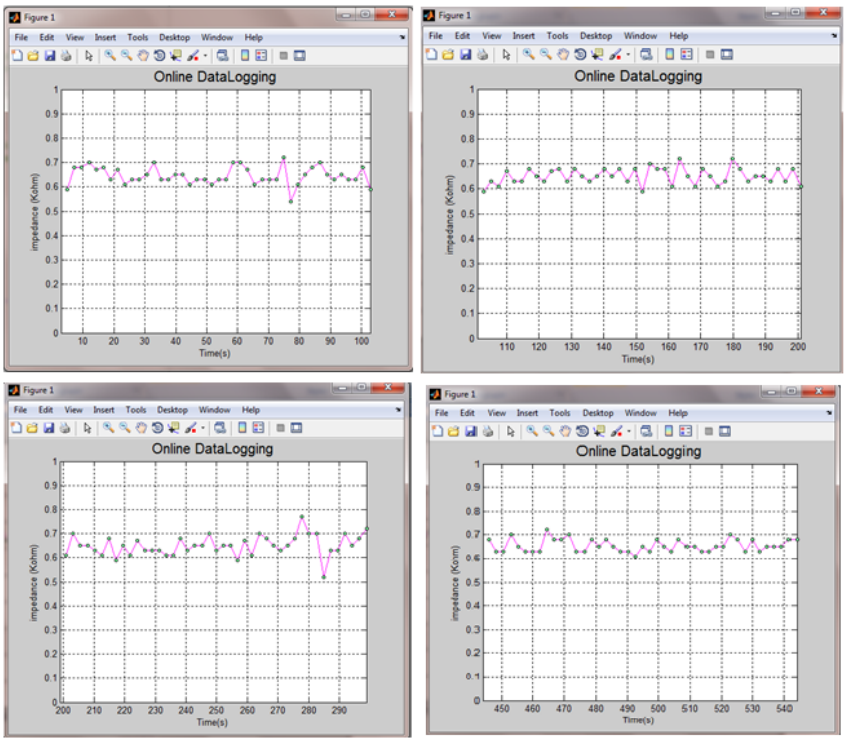

Figure 4.10 Output for continuous monitoring of pure goat blood.

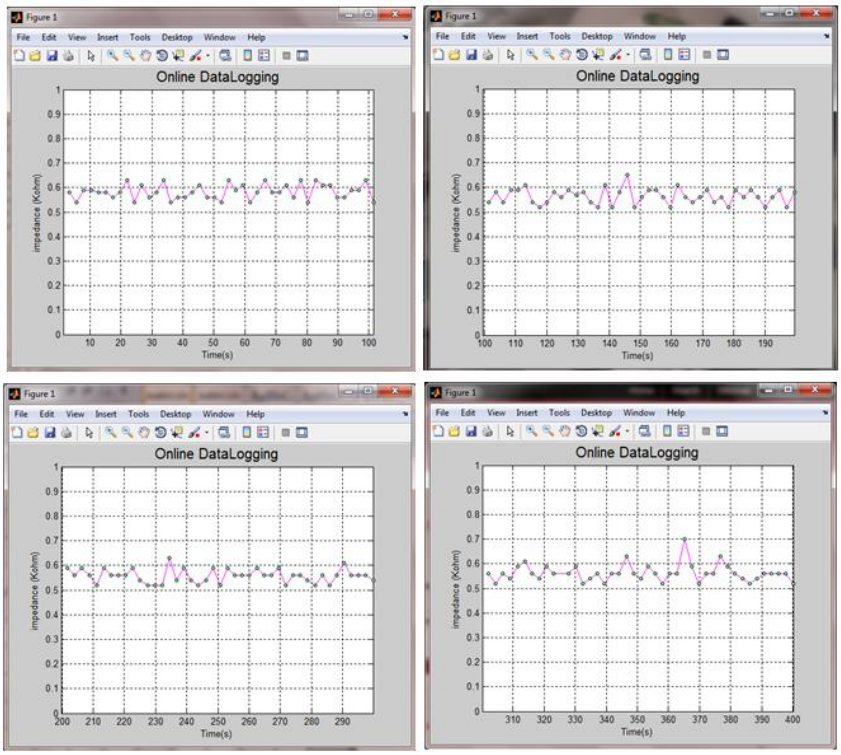

Figure 4.11 Output for continuous monitoring of diluted blood (blood and saline diluted in 4:1 ratio)

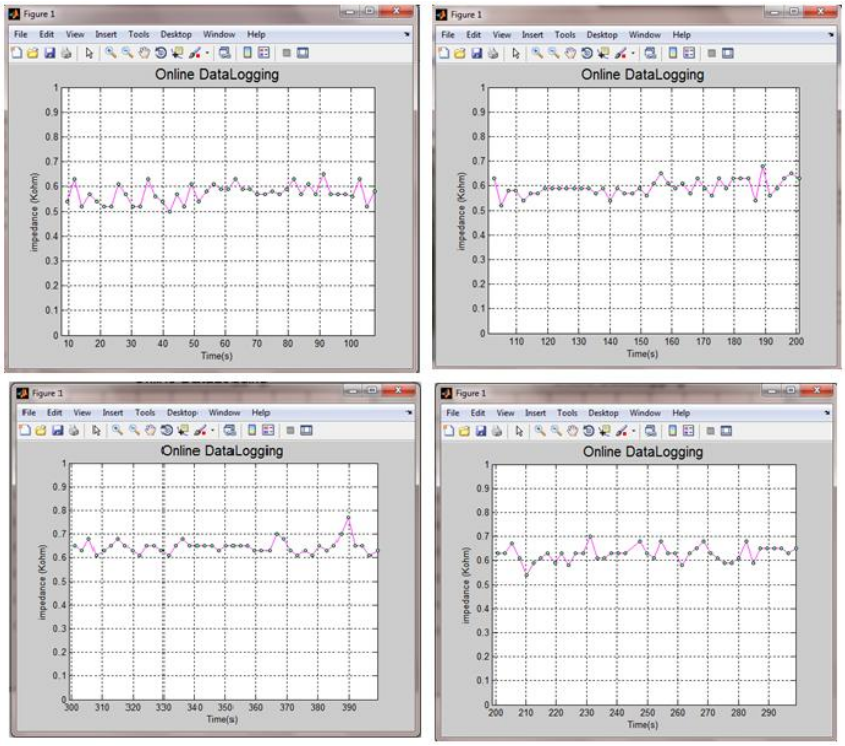

Figure 4.12 Output for continuous monitoring of diluted blood (blood and saline diluted in 1:4 ratio)

Chapter V

Conclusion

5. Conclusion

The impedance analyzer used now-a-days are much costlier and bulky. Developed bioimpedance analyzer is of low cost than the premium analyzers. But the accuracy in the results is almost same for both the devices. The developed bioimpedance analyzer also can be used for extracting physiological features like heart rate, stroke volume etc. for that we have to incorporate a instrumentation amplifier into the proposed circuit design in order to match the higher body impedance.

References

[1] F. Seoane, *et al.*, "Current source for multifrequency broadband electrical bioimpedance spectroscopy systems. A novel approach," in *Engineering in Medicine and Biology Society, 2006. EMBS'06. 28th Annual International Conference of the IEEE*, 2006, pp. 5121-5125.

[2] G. Walter, "A review of impedance plot methods used for corrosion performance analysis of painted metals," *Corrosion Science,* vol. 26, pp. 681-703, 1986.

[3] M.-C. Cho, *et al.*, "A bio-impedance measurement system for portable monitoring of heart rate and pulse wave velocity using small body area," in *Circuits and Systems, 2009. ISCAS 2009. IEEE International Symposium on*, 2009, pp. 3106-3109.

[4] U. G. Kyle, *et al.*, "Bioelectrical impedance analysis—part I: review of principles and methods," *Clinical Nutrition,* vol. 23, pp. 1226-1243, 2004.

[5] M. H. Oliver, *et al.*, "A rapid and convenient assay for counting cells cultured in microwell plates: application for assessment of growth factors," *Journal of cell science,* vol. 92, pp. 513-518, 1989.

[6] C.-G. Song, *et al.*, "Comparison of bio-impedance changes and EMG activity during daily events," in *Biomedical Circuits and Systems Conference, 2008. BioCAS 2008. IEEE*, 2008, pp. 369-372.

[7] Y. N. Kalia, *et al.*, "Iontophoretic drug delivery," *Advanced drug delivery reviews,* vol. 56, pp. 619-658, 2004.

[8] S. Gabriel, *et al.*, "The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz," *Physics in medicine and biology,* vol. 41, p. 2251, 1996.

[9] L. Yang and R. Bashir, "Electrical/electrochemical impedance for rapid detection of foodborne pathogenic bacteria," *Biotechnology advances,* vol. 26, pp. 135-150, 2008.

[10] J. W. Lee, *et al.*, "Precision constant current source for electrical impedance tomography," in *Engineering in Medicine and Biology Society, 2003. Proceedings of the 25th Annual International Conference of the IEEE*, 2003, pp. 1066-1069.

[11] A. Fabre, *et al.*, "High frequency applications based on a new current controlled conveyor," *Circuits and Systems I: Fundamental Theory and Applications, IEEE Transactions on,* vol. 43, pp. 82-91, 1996.

[12] M. Wang, *et al.*, "A high-performance EIT system," *Sensors Journal, IEEE,* vol. 5, pp. 289-299, 2005.

[13] D. Chen, *et al.*, "The dynamic response of a Butterworth low-pass filter in an ac-based electrical capacitance tomography system," *Measurement Science and Technology,* vol. 21, p. 105505, 2010.

[14] J. Webster, *Medical instrumentation: application and design*: John Wiley & Sons, 2009.

[15] D. Biswas, *et al.*, "Development of low cost bioimpedance analyser for analysing various biological samples," in *Communications, Devices and Intelligent Systems (CODIS), 2012 International Conference on*, 2012, pp. 508-511.

[16] M. P. Patel, *et al.*, "Delivery of macromolecules across oral mucosa from polymeric hydrogels is enhanced by electrophoresis (iontophoresis)," *Dental Materials,* vol. 29, pp. e299-e307, 2013.

[17] D. Satapathy, *et al.*, "Sunflower-oil-based lecithin organogels as matrices for controlled drug delivery," *Journal of Applied Polymer Science,* vol. 129, pp. 585-594, 2013.

[18] S. S. Sagiri, *et al.*, "Effect of composition on the properties of tween-80–span-80-based organogels," *Designed Monomers and Polymers,* vol. 15, pp. 253-273, 2012.

[19] E. L. Thomas, "Myeloperoxidase, hydrogen peroxide, chloride antimicrobial system: nitrogen-chlorine derivatives of bacterial components in bactericidal action against Escherichia coli," *Infection and Immunity,* vol. 23, pp. 522-531, 1979.

[20] H. Bai, *et al.*, "Biosynthesis of cadmium sulfide nanoparticles by photosynthetic bacteria< i> Rhodopseudomonas palustris</i>," *Colloids and surfaces B: Biointerfaces,* vol. 70, pp. 142-146, 2009.

[21] P. Pecoraro, *et al.*, "Body mass index and skinfold thickness versus bioimpedance analysis: fat mass prediction in children," *Acta diabetologica,* vol. 40, pp. s278-s281, 2003.